

We claim:

- 1) A *Mycobacterium* strain with a modified tyrosine phosphatase gene in its genome, wherein the said *Mycobacterium* strain is incapable of expressing the active tyrosine phosphatase gene.
- 5 2) The *Mycobacterium* strain as claimed in claim 1 wherein the *Mycobacterium* species is selected from a group consisting of *M. tuberculosis* and *M. bovis*.
- 3) The *Mycobacterium* strain as claimed in claim 1 wherein the modified tyrosine phosphatase gene is modified *mptpA* gene.
- 4) The *Mycobacterium* strain as claimed in claim 3 wherein the modified *mptpA* gene is as shown in SEQ ID NO : 15.
- 10 5) The *Mycobacterium* strain as claimed in claim 1 wherein the modified tyrosine phosphatase gene is modified *mptpB* gene.
- 6) The *Mycobacterium* strain as claimed in claim 5 wherein the modified *mptpB* gene is as shown in SEQ ID NO : 16.
- 15 7) A recombinant vector comprising the modified *mptpA* gene of claim 3.
- 8) A recombinant vector as claimed in claim 7 is pAKΔA.
- 9) A recombinant vector comprising the modified *mptpB* gene of claim 5.
- 10) A recombinant vector as claimed in claim 9 is pBKΔB.
- 11) The recombinant vector as claimed in claim 7, wherein the nucleotide sequence of *mptpA* gene as shown in **SEQ ID NO: 11** is modified.
- 20 12) The recombinant vector as claimed in claim 9, wherein the nucleotide sequence of *mptpB* gene as shown in **SEQ ID NO: 12** is modified.
- 13) The recombinant vector as claimed in claim 7 or 9, wherein the *mptpA* or *mptpB* gene is modified by insertion, deletion, mutation or substitution.
- 25 14) The recombinant vector as claimed in claim 7 or 9, wherein the *mptpA* or *mptpB* gene is modified by substituting an internal region of the *mptpA* or *mptpB* gene by an antibiotic resistance marker gene.
- 15) The recombinant vector as claimed in claim 14, wherein the antibiotic resistance marker gene imparts resistance to either hygromycin or chloramphenicol preferably to hygromycin.
- 30 16) The recombinant vector as claimed in claim 7 or 9, wherein a second antibiotic marker gene is inserted in the backbone of the said recombinant vector.
- 17) The recombinant vector as claimed in claim 16, wherein the second antibiotic marker gene imparts resistance to kanamycin or gentamycin.
- 35 18) An isolated nucleotide sequence of the *mptpA* gene encoding the mycobacterial tyrosine phosphatase A as shown in SEQ ID NO : 11.

- 19) An isolated nucleotide sequence of the *mptpB* gene encoding the mycobacterial tyrosine phosphatase B as shown in SEQ ID NO : 12.
- 20) An isolated nucleotide sequence of the modified *mptpA* gene as shown in SEQ ID NO : 15.
- 5 21) An isolated nucleotide sequence of the modified *mptpB* gene as shown in SEQ ID NO : 16.
- 22) A method for developing a *Mycobacterium* strain with a modified tyrosine phosphatase gene in its genome comprising the following steps:
- 10 a. extracting genomic DNA from *Mycobacterium* strain,
- b. amplifying the tyrosine phosphatase gene along with the flanking sequences using specific primers from the genomic DNA of step (a) to obtain a DNA fragment,
- c. characterizing the fragment of step (b),
- d. cloning the fragment of step (b) in a non-replicative vector,
- 15 e. modifying the fragment in the non-replicative vector of step (d),
- f. inserting an antibiotic resistance marker gene within the fragment of step (e) to obtain a non-replicative vector containing a modified tyrosine phosphatase gene,
- g. cloning of a second antibiotic resistance marker gene in the backbone of the non-replicative vector of step (f), to obtain a recombinant vector,
- 20 h. introducing the recombinant vector of step (g) into *Mycobacterium* strains,
- i. selecting for primary recombinant *Mycobacterium* strains using the first antibiotic selection marker gene,
- 25 j. culturing the primary recombinant *Mycobacterium* strains of step (i) harboring the first antibiotic resistance marker gene,
- k. selecting the secondary recombinant *Mycobacterium* strains of step (j) that is sensitive to the second antibiotic resistance gene present in the vector backbone,
- 30 l. culturing the secondary recombinant *Mycobacterium* strains of step (k), wherein the said recombinant *Mycobacterium* strain harboring the modified tyrosine phosphatase gene which shows defective growth in activated macrophages and animals.
- 35 23) The method as claimed in claim 22, wherein the *Mycobacterium* species is selected from a group consisting of *M. tuberculosis* and *M. bovis*.

- 24) The method as claimed in claim 22, wherein in step (b) the specific primers are selected from a group comprising of SEQ ID NO : 1 to 4 for amplification of *mtpA* along with its flanking regions and SEQ ID NO : 5 to 8 for amplification of *mtpB* along with its flanking regions.
- 5 25) The method as claimed in claim 22, wherein in step (b) the tyrosine phosphatase gene is *mtpA* gene as shown in SEQ ID NO : 11.
- 26) The method as claimed in claim 22, wherein in step (b) the tyrosine phosphatase gene is *mtpB* gene as shown in SEQ ID NO : 12.
- 27) The method as claimed in claim 22, wherein in step (b) the DNA fragment is a
10 sequence as shown in SEQ ID NO : 13.
- 28) The method as claimed in claim 22, wherein in step (b) the DNA fragment is a sequence as shown in SEQ ID NO : 14.
- 29) The method as claimed in claim 22, wherein in step (c) the DNA fragment is characterized by sequencing and restriction enzyme analysis.
- 15 30) The method as claimed in claim 22, wherein in step (f) the modified tyrosine phosphatase gene is modified *mtpA* gene as shown in SEQ ID NO : 15.
- 31) The method as claimed in claim 22, wherein in step (f) the modified tyrosine phosphatase gene is modified *mtpB* gene as shown in SEQ ID NO : 16.
- 32) The method as claimed in claim 30 or 31, wherein the *mtpA* or *mtpB* gene is
20 modified by insertion, deletion, mutation or substitution.
- 33) The method as claimed in claim 30 or 31, wherein the *mtpA* or *mtpB* gene is modified by substituting an internal region of the *mtpA* or *mtpB* gene by an antibiotic resistance marker gene.
- 34) The method as claimed in claim 33, wherein the antibiotic resistance marker
25 gene imparts resistance to either hygromycin or chloramphenicol preferably to hygromycin.
- 35) The method as claimed in claim 22, wherein in step (g) the second antibiotic marker gene imparts resistance to kanamycin.
- 36) The method as claimed in claim 22, wherein in step (g) the recombinant vector
30 is either pAKΔA or pBKΔB.
- 37) The method as claimed in claim 22, wherein in step (h) the introduction of the vector is by either electroporation or phages.
- 38) The method as claimed in claim 22, wherein in step (i) the selection of primary
35 recombinant *Mycobacterium* strain is by using either hygromycin or chloramphenicol.

- 39) The method as claimed in claim 22, wherein in step (k) the selection of secondary recombinant *Mycobacterium* strain which are resistant to either hygromycin or chloramphenicol but sensitive to second antibiotic resistance marker (kanamycin).